REPORT

ACUTE TOXICITY STUDY IN *DAPHNIA MAGNA*

WITH

[redacted]

(SEMI-STATIC)

NOTOX Project 338772
NOTOX Substance 111834/B

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by [redacted]. Reproduction, issue or disclosure to third parties in any form is not permitted without prior written authorisation from the sponsor.

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Study Director
Ing. M.H.J. Migchielsen

Date: November 13, 2002

Management:
Ing. E.J. van de Waart M.Sc.
Head of Genetic & Ecotoxicology

Date: 15/11/2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/AUDITS	REPORTING DATES
on-site inspection(s) (Process)	
July 08 to 15, 2002 (Ecotoxicology) August 19 to 30, 2002 (Analytical support)	July 17, 2002 September 02, 2002
protocol inspection(s) (Study)	
July 04, 2002	July 04, 2002
report audit(s) (Study)	
November 05, 2002	November 05, 2002

Head of Quality Assurance — C.J. Mitchell B.Sc.

Date: 18-NOV-02.

SUMMARY

Acute Toxicity Study in *Daphnia magna* with [redacted]

The study procedures described in this report were based on the ISO International Standard 6341: "Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus – Acute toxicity test, Third edition, 1996-04-01. In addition, the procedures were designed to meet the test methods and validity criteria of the EEC directive 92/69, Part C: Methods for the determination of ecotoxicity, Publication No. L383, December 1992, C.2. "Acute Toxicity for *Daphnia*", and the OECD guideline No. 202 Part I: "*Daphnia sp.*, Acute Immobilisation Test", Adopted April 4, 1984.

The batch of [redacted] tested was a clear and colourless liquid consisting of two main components, i.e. 28.6% peroxidic compounds and 67% Dimethyl phtalate. [redacted] was completely miscible with test medium at the concentrations tested.

The project started with a static range-finding test with daphnia exposed to nominal [redacted] concentrations of 0.1 to 100 mg/l, increasing with a factor of 10. After 24 hours of exposure all organisms exposed to 100 mg/l became immobilised. No further immobilisation occurred during the remaining test period in any of the lower test concentrations. The EC_{50} was expected to be between 10 and 100 mg/l.
Analysis of the samples taken during the range-finding test of the simultaneously performed study with carp (NOTOX Project 338761) showed that the measured concentrations of both major components present in [redacted] decreased by more than 20% during the test period. It was decided to continue testing applying a semi-static test design with renewal of test solutions after 24 hours as concentrations did not decrease by more than 20% during the first 24-hour test period.

The range-finding test was followed by a semi-static final EC_{50} test with renewal of test solutions after 24 hours of exposure. In the final EC_{50} test Daphnia were exposed for a maximum of 48 hours to nominal concentrations of 10, 18, 32, 56 and 100 mg/l. A blank-control was also included. The test was performed in duplicate with 10 daphnia per vessel. Samples for analysis of actual exposure concentrations were taken from the freshly prepared solutions at the start and after 24 hours of exposure and from the 24-hour old solutions after 24 and 48 hours of exposure.

Analysis of the samples taken during the final test showed that the measured concentrations (based on both components) were in agreement with nominal in the freshly prepared solutions at the start of exposure (88-101%) and the freshly prepared solutions at 24 hours of exposure (93-98%). This indicated that preparation procedures were adequate and repeatable.
During the 24-hour periods, for and after renewal, the concentrations measured did not decrease by more than 20% below initial. In addition, the average exposure concentrations all remained well above 80% relative to nominal. Consequently, the calculated toxicity parameters were based on the nominal test concentrations.

In the control, no daphnia became immobilised or trapped at the surface of the water. Further, all test conditions (pH, oxygen and temperature) remained within the ranges prescribed by the protocol.

[redacted] did not induce acute immobilisation of *Daphnia magna* at nominally 18 mg/l after 48 hours of exposure (NOEC). Note that a maximum response of 10% is acceptable for the control and therefore not considered treatment related.

The 24h-EC_{50} was 45 mg/l with a 95% confidence interval between 40 and 51 mg/l.

The 48h-EC_{50} was 34 mg/l with a 95% confidence interval between 29 and 41 mg/l.

PREFACE

Sponsor	[redacted]
Study Monitor	Dr. C.L.J. Braun SHERA, Regulatory Affairs
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Aquatic Toxicology:	
Study Director	Ing. M.H.J. Migchielsen
Technical co-ordinator	Ing. B. van Wees
Analytical Chemistry:	
Principal Scientist	Dr. Ir. E. Baltussen
Study Plan	Start Project: July 04, 2002 Start first exposure: July 22, 2002 Completion last exposure: September 11, 2002 Completion Analysis: September 19, 2002 Draft report: November 06, 2002 Completion project: November 13, 2002

TEST SUBSTANCE

Identification Chemical name CAS RN	[redacted]
Description	Clear colourless liquid
Batch	1510-14
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2003
Density	Approx. 1160 $kg.m^{-3}$
Stability in water	Unknown

The sponsor is responsible for all test substance data unless determined by NOTOX.

PURPOSE

The purpose of the toxicity test was to evaluate the influence of [redacted] on the mobility of *Daphnia magna*.

GUIDELINES

The study procedures described in this report were based on the ISO International Standard 6341: "Water quality - Determination of the inhibition of the mobility of *Daphnia magna* Straus - Acute toxicity test, Third edition, 1996-04-01.

In addition, the procedures were designed to meet the test methods and validity of the following guidelines:

- European Economic Community (EEC), EEC directive 92/69, Part C: Methods for the determination of ecotoxicity, Publication No. L383, December 1992, C.2. "Acute Toxicity for *Daphnia*".
- Organization for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, guideline No. 202 Part I: "*Daphnia sp.*, Acute Immobilisation Test", Adopted April 4, 1984.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data. No data will be withdrawn without the sponsor's written consent.

DEFINITIONS

Immobilisation: those animals not able to swim within 15 seconds after gentle agitation of the test vessel are considered to be immobile.

EC_{50}: the concentration estimated to immobilise 50% of the daphnia after a defined period of exposure.

No Observed Effect Concentration (NOEC): is the highest tested concentration at which no effect (i.e. immobilisation) is recorded.

TEST SYSTEM

Species	*Daphnia magna* (Crustacea, Cladocera) (Straus, 1820)
Reason for selection	This system has been selected as an internationally accepted species.
Validity of batch	Frequent inspection of the cultures with respect to the number of young, appearance of young and parental daphnia and global feeding behaviour.
Characteristics	For the test selection of young daphnia with an age of < 24 hours.

BREEDING

Start of each batch	With new-born animals, i.e. less than 3 days old, by placing about 250 of them into 10 litres of medium in an all-glass culture vessel.
Maximum age of the cultures	4 weeks
Renewal of the cultures	After 7 days of cultivation half of the medium twice a week.
Temperature of medium	18-22°C, constant within ± 1°C
Feeding	Daily, a suspension of fresh water algae.
Medium	M7, as prescribed by Dr. Elendt-Schneider (Elendt, B.-P., 1990: Selenium deficiency in Crustacea. An ultrastructural approach to antennal damage in *Daphnia magna* Straus. Protoplasma 154, 25-33).

Composition of medium M7:

ISO-medium: the following chemicals (analytical grade) are dissolved in freshly prepared ultra-pure water (tap water purified by reverse osmosis (milli-RO); Millipore Corp., Bedford, Mass., USA) (mg/l):

$CaCl_2.2H_2O$	293.8
$MgSO_4.7H_2O$	123.3
$NaHCO_3$	64.8
KCl	5.8

Medium M7: trace elements, macro nutrients and vitamins are added to freshly prepared ISO-medium to reach the following concentrations:

Trace elements (mg/l):	B	0.125
	Fe	0.05
	Mn	0.025
	Li, Rb and Sr	0.0125
	Mo	0.0063
	Br	0.0025
	Cu	0.0016
	Zn	0.0063
	Co and I	0.0025
	Se	0.0010
	V	0.0003
	$Na_2EDTA.2H_2O$	2.5
Macro nutrients (mg/l):	$Na_2SiO_3.9H_2O$	10.0
	$NaNO_3$	0.27
	KH_2PO_4	0.14
	K_2HPO_4	0.18
Vitamins (µg/l):	Thiamine	75.0
	B_{12}	1.0
	Biotin	0.75

The hardness: 250 mg/l expressed as $CaCO_3$ and the pH: 8.0 ± 0.2 after aeration.

REFERENCE SUBSTANCE

This report includes the results of a reference test with potassium dichromate.

PREPARATION OF TEST SOLUTIONS

The standard test procedures required generation of test solutions, which should contain completely dissolved test substance concentrations or stable and homogeneous mixtures or dispersions. The testing of concentrations that disturb the test system should be prevented (e.g. film of the test substance on the water surface).

The batch of [redacted] tested was a clear and colourless liquid consisting of two main components, i.e. 28.9% peroxidic compounds and 66% Dimethyl phtalate (see also attached analysis certificate). [redacted] was completely miscible with test medium at the concentrations tested.

Preparation of test solutions started with stock solutions at nominally 100 mg/l. These solutions were magnetically stirred for 15 to 20 minutes following treatment with ultrasonic waves for 5 minutes during the range-finding test. The resulting, clear and colourless, stock solutions were then used to prepare the lower test concentrations by subsequent dilutions in test medium. Test solutions were renewed after 24 hours of exposure in the final test. Most test solutions used originated from the simultaneously performed study with carp (NOTOX Project 338761). The solutions of 32 and 56 mg/l were separately prepared for the renewal at 24 hours.

RANGE-FINDING TEST

A range-finding test was performed to provide information about the range of concentrations to be used in the final test. Daphnia were exposed for 48 hours to a concentration range of 0.1 to 100 mg/l forming a geometric progression with a factor of 10.

FINAL TEST:

TEST CONCENTRATIONS

[redacted]	10, 18, 32, 56 and 100 mg/l.
Controls	Test medium without test substance or other additives (0 mg/l).

TEST PROCEDURE AND CONDITIONS

Test type	Semi-static, with renewal after 24 hours.
Test duration	48 hours
Test vessels	100 ml, all-glass
Medium	ISO, prepared in milli-RO water
Number of daphnia	20 per concentration
Loading	10 per vessel containing 80 ml medium
Light	16 hours photoperiod daily
Feeding	No feeding
Aeration	No aeration of the test solutions.
Introduction of daphnia	Within 2 hours after preparation of the test solutions.

SAMPLING FOR ANALYSIS OF TEST CONCENTRATIONS

During the final EC_{50} test samples for analysis were taken from all test concentrations and the control according to the following sampling schedule:

Sampling:	Frequency	at t= 0 h (in combination with NOTOX Project 338761) and t= 24 h from freshly prepared solutions and at t= 24 h and t= 48 h from the 24h-old solutions.
	Volumes	0, 10 and 18 mg/l: 6 ml 32 mg/l: 3 ml 56 mg/l: 2 ml 100 mg/l: 1 ml (only from fresh t=0, and old t=24 hour samples)
	Storage	Not applicable, all samples were freshly analysed.

Additionally, reserve samples of 12 ml were taken from all test solutions. These samples were stored in a deep-freeze for possible analysis until delivery of the final report with a maximum of three months. The method of analysis is described in the appended Analytical Report.

MEASUREMENTS AND RECORDINGS

Immobility (including mortality)	At 24 hours and 48 hours.
pH and dissolved oxygen	At the beginning, after 24 hours of exposure and at the end of the test, for all concentrations and the control(s).
Temperature of medium	Daily in one control vessel, beginning at the start of the test.

DATA HANDLING

Calculation of EC_{50}:

The EC_{50}-value was calculated at 24 and 48 hours of exposure from the probits of the percentages of affected daphnia and the logarithms of the corresponding test substance concentrations using the maximum likelihood estimation method (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition).

RESULTS

Static range-finding test:

Table 1 shows the responses recorded during the range-finding test.

After 24 hours of exposure all organisms exposed to 100 mg/l became immobilised. No further immobilisation occurred during the remaining test period in any of the lower test concentrations. The EC_{50} was expected to be between 10 and 100 mg/l.

Table 1: Incidence of immobility in the range-finding test:

Concentration [redacted] **(mg/l)**	**Number Daphnia exposed**	**Response at 24 h***		**Response at 48 h**	
		number	**%**	**Number**	**%**
Blank-control	10	0	0	0	0
0.1	10	0	0	0	0
1.0	10	0 (1)	0	0	0
10	10	0 (3)	0	0	0
100	10	10	100	10	100

* Between brackets: number of daphnia trapped at the surface. These daphnia were reimmersed in the respective test solutions before recording of mobility.

Determination of the stability of [redacted] nder test conditions.

Analysis of actua [redacted] concentrations was based on the two major components present in [redacted] ndicated as MIPKP-T3 peak 1 and MIPKP-T3 peak 2). Analysis of the samples taken during the range-finding test of the simultaneously performed study with carp (NOTOX Project 338761) showed that the measured concentrations of both components decreased by more than 20% during the test period. It was decided to continue testing applying a semi-static test design with renewal of test solutions after 24 hours as concentrations did not decrease by more than 20% during the first 24-hour test period.

Final test:

The results of analysis of the samples taken during the final study are described in Tables 1 and 2 of the appended Analytical Report.

Analysis of the samples taken during the final test showed that the measured concentrations (based on both components) were in agreement with nominal in the freshly prepared solutions at the start of exposure (88-101%) and the freshly prepared solutions at 24 hours of exposure (93-98%). This indicated that preparation procedures were adequate and repeatable. During the 24-hour periods, for and after renewal, the concentrations measured did not decrease by more than 20% below initial. In addition, the average exposure concentrations all remained well above 80% relative to nominal. Consequently, the calculated toxicity parameters were based on the nominal test concentrations.

Immobility

Table 2 shows the responses recorded during the final EC_{50} test.

The responses recorded in this test allowed for reliable determination of an EC_{50}. The responses recorded were in agreement with what was expected based on the results of the range-finding test. Daphnids exposed to 100 mg/l were microscopically examined after 24 hours to confirm whether they were immobilised or dead. All daphnids proved to be dead. Consequently, these organisms were not transferred to freshly prepared test solutions.

Table 2: Acute immobilisation of daphnia after 24 and 48 hours in the final EC_{50}-test.

Concentration [redacted] (mg/l)	Vessel Number	Number Daphnia exposed	Response at 24 *		Response at 48 h	
			number	%	number	%
Blank-control	A	10	0	0	0	0
	B	10	0	0	0	0
10	A	10	0	0	0	0
	B	10	0	0	0	0
18	A	10	0	0	1	10
	B	10	0	0	0	0
32	A	10	1	10	3	30
	B	10	0	0	5	50
56	A	10	9 (3)	90	9	90
	B	10	8 (1)	80	9	90
100	A	10	10	100	10	100
	B	10	10	100	10	100

* Between brackets: number of daphnids observed trapped at the surface.

Experimental conditions

The results of measurement of pH and oxygen concentrations (mg/l) are presented in Table 3.

The temperature of the test medium measured in the blank-control varied from 20.3 to 20.4°C.

Table 3: pH and oxygen concentrations during the final test.

Concentration [redacted] (mg/l)	Start (t=0 h)		t=24h old		t=24h fresh		End (t=48 h)	
	pH	O_2	pH	O_2	pH	O_2	pH	O_2
Blank-control	7.7	8.6	7.9	8.7	7.7	8.5	7.9	8.7
10	7.7	8.6	7.8	8.7	7.7	8.6	7.8	8.8
18	7.7	8.6	7.8	8.8	7.7	8.7	7.8	8.8
32	7.7	8.6	7.8	8.8	7.9	8.6	7.8	8.8
56	7.7	8.6	7.8	8.7	7.8	8.6	7.8	8.9
100	7.7	8.7	7.9	8.8	-	-	-	-

ACCEPTABILITY OF THE TEST

1. In the controls, no Daphnia became immobilised or trapped at the surface of the water.
2. The analytical program showed that the actual test concentrations were maintained at more than 80 % of the initial concentration.
3. Further, all test conditions (pH, oxygen concentration and temperature) remained within the ranges prescribed by the protocol.

CONCLUSION

Under the conditions of the present study [redacted] did not induce acute immobilisation of *Daphnia magna* at nominally 18 mg/l after 48 hours of exposure (NOEC). Note that a maximum response of 10% is acceptable for the control and therefore not considered treatment related.

The 24h-EC_{50} was 45 mg/l with a 95% confidence interval between 40 and 51 mg/l.

The 48h-EC_{50} was 34 mg/l with a 95% confidence interval between 29 and 41 mg/l.

See Tables 4 and 5 and Figures 1 and 2.

Table 4: EC_{50} value at 24 hours and related parameters.

```
24h-EC50 Daphnia = 44.8 mg/l
95 % fiducial limits: 39.7 - 50.6 mg/l

index of regression significance: g=0.16
chi-squared=1.45, with 4 degrees of freedom

regression line: log10(conc.)=1.65+(probit-4.99)/10.70
```

conc. mg/l	group size	response	corrected fraction	expected fraction	chi2
32	10	1	0.10	0.05	0.54
32	10	0	0.00	0.05	0.52
56	10	9	0.90	0.85	0.19
56	10	8	0.80	0.85	0.20
100	10	10	1.00	1.00	0.00
100	10	10	1.00	1.00	0.00
					1.45

Figure 1: Percentage response (=immobility) of Daphnia magna as function of the log concentration of [redacted] at 24h.

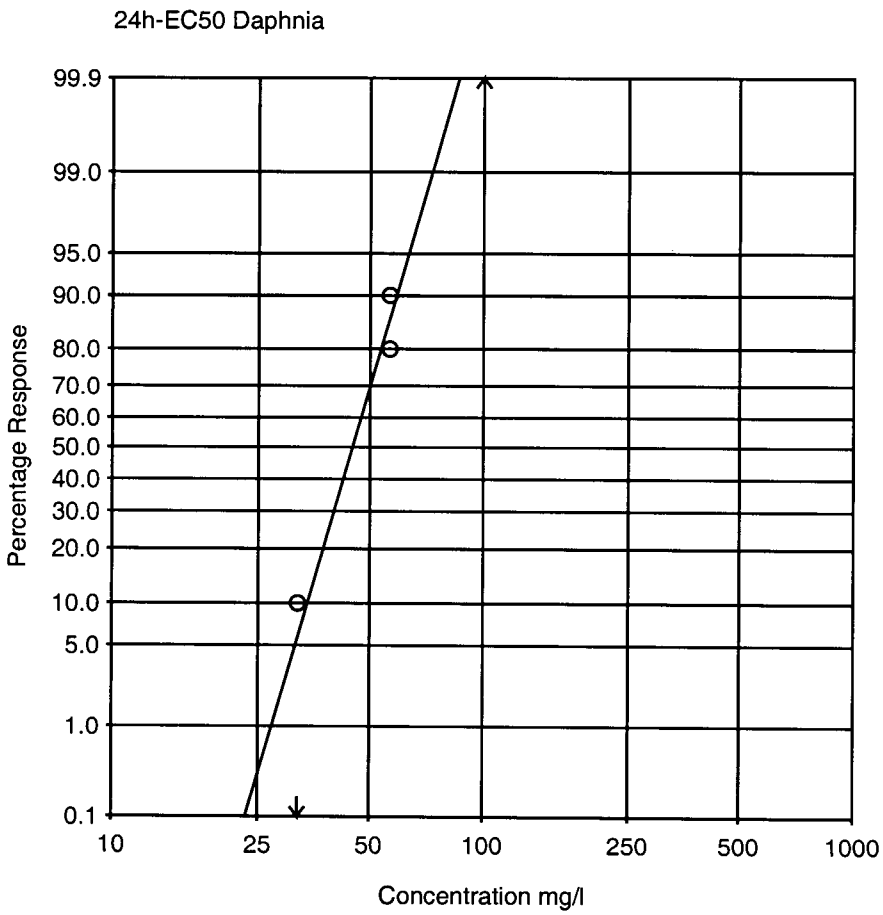


Table 5: EC_{50} value at 48 hours and related parameters.

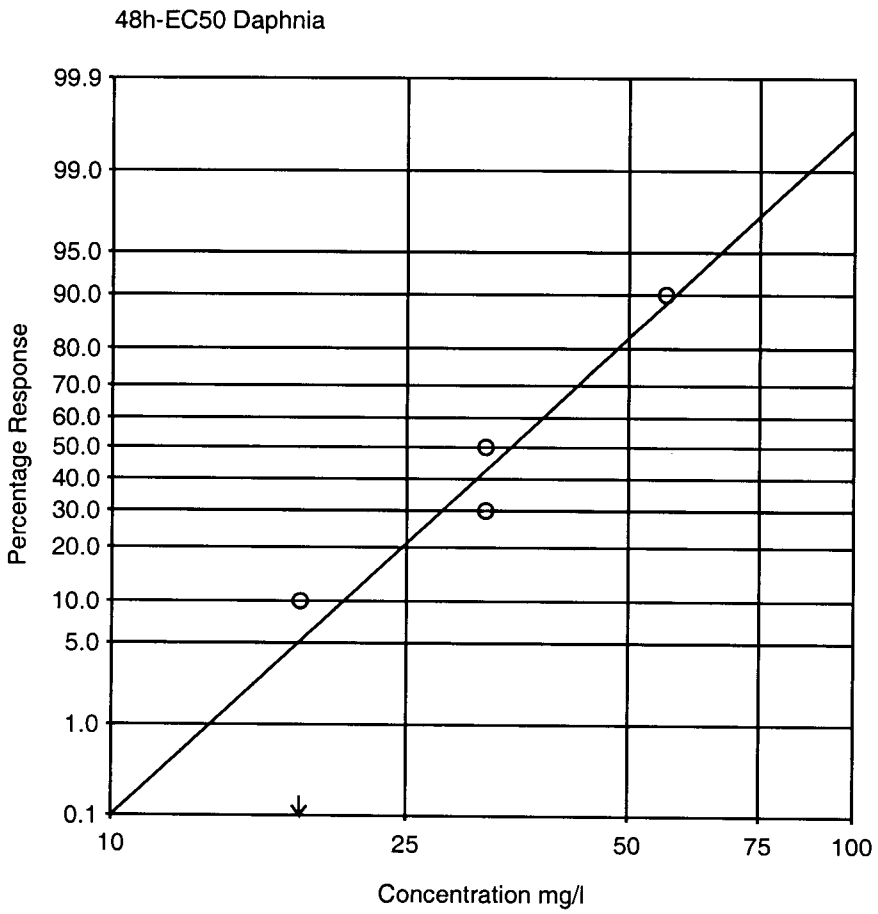
48h-EC50 Daphnia = 34.4 mg/l
95 % fiducial limits: 29.3 - 41.1 mg/l

index of regression significance: g=0.15
chi-squared=2.20, with 4 degrees of freedom

regression line: log10(conc.)=1.52+(probit-4.89)/5.75

conc. mg/l	group size	response	corrected fraction	expected fraction	chi2
18	10	1	0.10	0.04	0.86
18	10	0	0.00	0.04	0.43
32	10	3	0.30	0.43	0.67
32	10	5	0.50	0.43	0.21
56	10	9	0.90	0.89	0.01
56	10	9	0.90	0.89	0.01
					2.20

Figure 2: Percentage response (=immobility) of Daphnia magna as function of the log concentration of [redacted] at 48h.



REFERENCE TEST

Start: August 05, 2002
End : August 07, 2002

48-hour Acute Toxicity Study in *Daphnia magna* with $K_2Cr_2O_7$ (NOTOX Project 356658).

The study procedures described in this report were based on the ISO International Standard 6341, the EEC directive 92/69, Part C.2. "Acute toxicity for *Daphnia*" and the OECD guideline No. 202: "Daphnia sp., Acute Immobilisation Test", Adopted April 4, 1984.

The reference test was carried out to check the sensitivity of the test system as used by NOTOX. Daphnia were exposed for a maximum of 48 hours to $K_2Cr_2O_7$ concentrations of 0.10, 0.18, 0.32, 0.56, 1.0 and 1.8 mg/l and to a blank control. Ten daphnia were exposed per concentration.
The reference substance, potassium dichromate ($K_2Cr_2O_7$, art. 4864, batch no. K28974764) was obtained from Merck, Darmstadt, Germany.

Acute immobilization of daphnia after 24 and 48 hours in the reference test with potassium dichromate:

Concentration (mg/l)	**Number Exposed**	**% immobile**		**Expected response (%) After 48 hours** [1]	
		24h	**48h**	**Minimal**	**Maximal**
Blank-control	10	0	0	0	10[2]
0.10	10	0	0	0	10
0.18	10	0	0	0	10
0.32	10	0	0	0	30
0.56	10	0	10	0	100
1.0	10	0	50	40	100
1.8	10	100	100	100	100

[1] Based on historical data of the previous years (n>60).
[2] A maximum response of 10% does not invalidate the results of the test.

The actual responses in this reference test with $K_2Cr_2O_7$ are within the ranges of the expected responses at the different concentrations. Hence, the sensitivity of this batch of *D. magna* was in agreement with the historical data collected at NOTOX.

The 24h-EC_{50} was 1.3 mg/l with 0% immobility at 1.0 mg/l and 100% immobility at 1.8 mg/l.

The 48h-EC_{50} was 0.90 mg/l with 95% fiducial limits of 0.75 – 1.2 mg/l.

The raw data from this study are kept in the NOTOX archives. The test described above was performed under GLP.

CERTIFICATE OF ANALYSIS

[redacted]

Certificate of Analysis [redacted]

TNA-2001007
page 1 of 2

ICS-331

Product name :	[redacted]
Chemical name :	
Batch number :	**1510-14**

Test results:

Method	Analysis of	Unit	Result *[1]
Jo/72.11, Jo/95.2	Peroxidic compounds (sum) ***See page 2 for a specification***	% m/m	28.6 (± 1.5)
J20010792	[redacted]	% m/m	67.0 (± 1.0)
J20010792	[redacted]	% m/m	2.0 (± 0.3)
Amp/88.9	Water	% m/m	2.6 (± 0.3)
J20010792	Unidentified impurities	% m/m	0.5 (± 0.2)

*[1] bracketed values are estimated 95% confidence intervals

File code : **TNA-2001007**

Analytical documentation : 20010792

[redacted]

CERTIFICATE OF ANALYSIS

[redacted]

Certificate of Analysis [redacted]

[redacted]atch 1510-14: specification of the peroxidic compounds

structure	% m/m
[redacted]	

[redacted]

ANALYTICAL REPORT

ACUTE TOXICITY STUDY IN *DAPHNIA MAGNA*

WITH

[redacted]

(SEMI-STATIC);

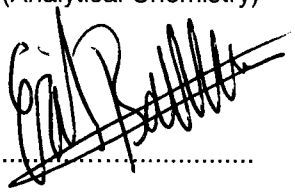
DETERMINATION OF THE CONCENTRATIONS

NOTOX Project 338772
NOTOX Substance 111834/B

REPORT APPROVAL

PRINCIPAL SCIENTIST:

Dr. Ir. E. Baltussen
(Analytical Chemistry)



Date: 07 NOV 2002

PREFACE

Study plan (analytical study)	Start: 12 September 2002 Completed: 19 September 2002

PURPOSE

The purpose of the analytical study was to determine the test concentrations.

REAGENTS

Acetonitrile	HPLC-grade, Labscan, Dublin, Ireland
Milli-Q water	Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges; Millipore, Bedford, MA, USA
ISO-medium	see main report

SAMPLE PRETREATMENT

All samples were stored in a deep freeze. On the day of analysis, the samples were defrosted at room temperature.

The entire volume of each sample was transferred quantitatively into a 6 ml vial. If necessary, the vials were filled up to 6 ml with ISO-medium to obtain concentrations within the calibration range.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC CONDITIONS

Quantitative analyses were based on the area of two peaks (MIPKP-T3 peak 1 and MIPKP-T3 peak 2) with retention times of 13.6 and 14.5 minutes in the HPLC chromatogram of [redacted] (See NOTOX Project 352968: "Implementation and validation of an analytical method for [redacted]).

Analytical conditions

A SPE-LC method was implemented and validated under Notox Project 352968. This method was based on a Zorbax RX-C18 column using a gradient of acetonitrile and Milli-Q water as the mobile phase, a column temperature of 25°C and a spectrophotometric detector set to read the absorbance at 220 nm.

Standard and calibration solutions

Standard solutions of [redacted] were prepared in acetonitrile.

Calibration solutions in ISO-medium were made up from two standard solutions.

DATA HANDLING

General

Mean:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

where
x_i = measured value
n = number of measurements

Maximum deviation:

[(highest value - lowest value)/mean] * 100%
where 'mean' is the mean value of the highest and the lowest value.

Calibration

Response:

R = Peak area test substance [units]

Calibration curve:

The response was correlated with the concentration test substance, using linear regression analysis (least squares method; weighting factor (1/concentration) was used)).

$$R = a * C + b$$

R = response calibration solution [units]
C = concentration of test substance in calibration solution [mg/l]
a = slope [l/mg]
b = intercept [units]

During analysis, calibration curves were constructed using six concentrations. For each concentration, two responses were used. The coefficient of correlation was > 0.99.

Samples

Concentration of [redacted] analysed in the samples:

$$C = \frac{(R-b) * d}{a} \text{ [mg/l]}$$

R = response sample [units]
d = dilution factor
a = slope [units*l/mg]
b = intercept [units]

Relative to nominal concentration:

$$\frac{\text{Concentration analysed}}{\text{Concentration nominal}} * 100 \text{ [\%]}$$

RESULTS

Tables 1-2 show the analytical results of this study*.

Table 1 Concentrations in test medium based on [redacted] peak 1 (final test).

Time of sampling [hours]	Date of sampling [dd-mm-yy]	Date of analysis [2] [dd-mm-yy]	Concentration		
			Nominal [mg/l]	Analysed [1] [mg/l]	Relative to nominal [%]
0 (fresh)	09-09-02	13-09-02 [4]	0	n.d.	n.a.
			10	8.81	88
		26-09-02 [4]	18	16.1	89
			32	28.5	89
			56	50.9	91
			100	92.0	92
24(fresh)	10-09-02	19-09-02 [3]	0	n.d.	n.a.
			18	16.7	93
			32	30.0	94
			56	51.8	93
24 (old)	10-09-02	12-09-02	0	n.d.	n.a.
			10	8.39	84
			18	15.6	87
			32	27.6	86
			56	48.7	87
			100	89.9	90
48 (old)	11-09-02	19-09-02 [3]	0	n.d.	n.a.
			18	16.2	90
			32	29.3	91
			56	50.8	91

[1] Mean of duplicate samples. The maximum deviation between the responses was calculated for each sample and was < 10%.

[2] Samples were frozen until analysis.

[3] Samples were frozen until pre-treatment on 17-09-02 and analysed on 19-09-02 after storage in the autosampler due to analytical problems. The samples were found to be stable during storage.

[4] Analysed during NOTOX project 338761: "96-Hour acute toxicity study in carp with Trigonox R-938 (semi-static)".

n.d. Not detected.

n.a. Not applicable.

* All recoveries and relative values were calculated using not-rounded concentrations. Therefore, some differences might be observed when calculating the recoveries and relative values using the concentrations as mentioned in the table.

Table 2 Concentrations in test medium based on MIPKP-T3 peak 2 (final test).

Time of sampling [hours]	Date of sampling [dd-mm-yy]	Date of analysis [2] [dd-mm-yy]	Concentration		
			Nominal [mg/l]	Analysed [1] [mg/l]	Relative to nominal [%]
0 (fresh)	09-09-02	13-09-02 [4]	0	n.d.	n.a.
			10	9.58	96
		26-09-02 [4]	18	17.8	99
			32	31.4	98
			56	55.2	99
			100	101	101
24(fresh)	10-09-02	19-09-02 [3]	0	n.d.	n.a.
			18	17.3	96
			32	31.4	98
			56	54.1	97
24 (old)	10-09-02	12-09-02	0	n.d.	n.a.
			10	9.05	90
			18	16.9	94
			32	29.7	93
			56	52.3	93
			100	97.1	97
48 (old)	11-09-02	19-09-02 [3]	0	n.d.	n.a.
			18	16.6	92
			32	30.3	95
			56	52.6	94

[1] Mean of duplicate samples. The maximum deviation between the responses was calculated for each sample and was < 10%.

[2] Samples were frozen until analysis.

[3] Samples were frozen until pre-treatment on 17-09-02 and analysed on 19-09-02 after storage in the autosampler due to analytical problems. The samples were found to be stable during storage.

[4] Analysed during NOTOX project 338761: "96-Hour acute toxicity study in carp with Trigonox R-938 (semi-static)".

n.d. Not detected.

n.a. Not applicable.